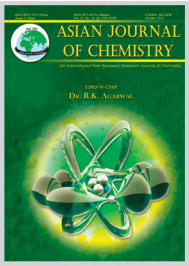
Asian Journal of Chemistry; Vol. 24, No. 8 (2012), 3396-3400

ASIAN JOURNAL OF CHEMISTRY

www.asianjournalofchemistry.co.in



Determination of Quercetin in Medicinal Plants Such as Rose Hip (*Rosa canina*), Bettle (*Urtica dioica*), Terebinth (*Terebinthina chica*) and Purslane (*Portulace oleracea*) using HPLC-MS Method

CEMILE OZCAN[1], YUSUF DILGIN[2] and MEHMET YAMAN[3,*]

[1]Department of Chemistry, Science and Arts Faculty, Kirklareli University, Kirklareli, Turkey
[2]Department of Chemistry, Science and Arts Faculty, Canakkale Onsekiz Mart University, Canakkale, Turkey
[3]Chemistry Department, Sciences Faculty, Firat University, Elazig, Turkey

*Corresponding author: Fax: +90 424 2330062; Tel.: +90 424 2370000/3684; E-mail: ijpacmy@gmail.com; myaman@firat.edu.tr

(*Received*: 1 July 2011; *Accepted*: 6 March 2012) AJC-11151

A simple and rapid high performance liquid chromatography-mass spectrometry (HPLC-MS) method was developed for the determination of quercetin in four plants and fruits samples which are rose hip (*Rosa canina*), nettle (*Urtica dioica*), terebinth (*Terebinthina chica*) and purslane (*Portulace oleracea*). For this purpose, flow rate of mobile phase, fragmentor potential, injection volume and column temperature were optimized as 0.6 mL min^{-1}, 140 V, 5 µL and 30 ºC, respectively. While the average recoveries were found at least 90 %, limit of detection and limit of quantification were found to be 0.1 and 0.4 mg L^{-1}, respectively. The relative standard deviation for retention time and peak area of 25 mg L^{-1} quercetin was found to be 2.4 % and 3.5 %, respectively. The optimized method was successfully applied to determination of quercetin in the plant and fruits samples after their extraction with a mixture of HCl, methanol and ascorbic acid.

Key Words: HPLC-MS, Quercetin, Rose hip, Nettle, Purslane, Terebinth, Flavonoids.

INTRODUCTION

An important group of phenolic compounds, flavonoids, are widely distributed throughout the plant kingdom. A number of plants, fruits and vegetables include these important compounds, but their contents varies from plant to plant or even different organs of same plant such as flower, root, leaves and body. These compounds are generally responsible for the attractive colors of flowers, fruits and leaves[1-3]. It is well known that these natural compounds supply beneficial effects on health due to their extensive biological activities including anticancer[4], anti-HIV[5], antiinflammatory[6], antiviral[7], antibacterial[8] *etc*. The epidemiological studies have indicated that the regular consumption of foods including rich phenolic compounds such as fruits, vegetables, whole grain cereals, red wine, tea, *etc*. shows a preventive role for cardiovascular diseases, neurodegenerative diseases and certain cancers[9-11]. Flavonoids show a powerful antioxidant activity by several ways including; 1) direct trapping of reactive oxygen species; 2) inhibition of enzymes responsible for superoxide anion production; 3) chelating of transition metals involved in processes forming radicals and 4) prevention of peroxidaiton process by reducing alkoxyl and peroxyl radicals[12-14]. Therefore, the development of analytical methods for sensitive detection and structural characterization of these compounds has received considerable attention due to their importance for health.

Among the flavonoids, quercetin (3,3, 4',5,7-pentahydroxylflavone, QU), has a great importance due to its strong antioxidant effect which can be attributed to the high number of hydroxyl substituent groups and to its conjugated *p* orbitals by which quercetin is able to donate electrons or hydrogens. Moreover, it is one of the most abundant dietary and also frequently studied flavonoids. In plants, it occurs mainly in leaves and in the other parts of the plants as aglycones and glycosides, in which one or more sugar groups such as glucose, galactose, rhamnose is bound to phenolic groups by glycosidic bonds. In general, quercetin-glycosides contain a sugar group at the 3-position. Glycosylation utilizes to increment in the polarity of the flavonoid molecule, which is necessary for storage in plant cell vacuoles[15]. The main dietary sources of quercetin are vegetables, fruits and red wine. Food derived quercetin could be presented in its glysocides form and thus the effectiveness of its antioxidant activity can be changed by the position of the sugar group. Quercetin aglycone seems to be a more active chain-breaking antioxidant than its glycoside counterparts because of its higher accessibility to the site of chain-initiating and chain-propagating free radicals in membranous phospholipid bilayers[4].

This important flavonoid displays a variety of biological activities including cardiovascular protection, anticancer activity, antiulcer effects, antiallergy activity, cataract prevention,

antiviral activity and antiinflammatory effects[16]. It is known that the antioxidants in natural products have more effect than the unnatural products like synthetic cancer drugs. It was reported that whereas synthetic cancer drugs cause non-specific killing of cells, natural products offer protective and therapeutic actions to all cells with low cytotoxicity and are beneficial in producing nutrient repletion to compromised people[17]. Therefore, the detection of quercetin in natural products has received a great deal of attention due to its important functions on health. Thus, many scientists have been focused on the development of a selective, simple, cheap and accurate method for its determination. So far, the determination of quercetin has been accomplished by using chromatographic[18-28], electrochemical[29,30] and spectrophotometric[31,32] methods. Among them, chromatographic methods were extensively preferred since they provide means of low detection limit with a relatively free matrix effect as well as quercetin and its glycosides can be easily monitored with these methods. These methods were used with different detection systems such as gas chromatography-mass spectrometry (GC-MS)[18], high-performance liquid chromatography-mass spectrometry (HPLC-MS)[19-22], ultraviolet detection (HPLC-UV)[23-25], diode array detection (HPLC-DAD)[20,26], fluorimetric[25], chemiluminescence[27] and electrometric detection[23,25] and capillary electrophoresis[28].

Sample preparation is an important procedure in determination of flavonoids in natural samples. Although, the same chromatographic method and detection mode have been used for determination of a flavonoid in natural compounds in many studies, different concentrations were reported because of the different sample preparation techniques such as liquid-liquid extraction using various solvents or solvent mixtures, solid-phase extraction using different adsorbents. For example, Aguirre-Hernández *et al.*[19] studied the analysis of quercetin and kaempferol in the tilla species which is a medicinal plant using HPLC-MS system. In extraction of plant materials, 2 kg air dried powdered samples were extracted with hexane (4 L × 3), ethyl acetate (4 L × 3) and methanol (4 L × 3) by maceration at room temperature (22 ºC). The solvents were separated from the residues by gravity filtration and then evaporated in vacuum. Yang *et al.*[20] used 5 mL % 70 methanol aqueous solution containing 0.1 % HCl (pH 2.08) for the extraction of 1 g of frozen petel powder. Then, they performed simultaneous determination of anthocyanines and flavonols in this sample using HPLC-DAD/electrospray ionization-mass spectrometry. In another sample preparation procedure, an aliquot of 40 mL 80 % methanol, 10 mL 6 M HCl and 80 mg ascorbic acid for 1 g of samples were used for extraction of flavonoids in 20 selected vegetables[21]. After the complete extraction, the flavonoid contents were analyzed with HPLC-MS. Quercetin was found in collard greens, mustard greens, kale, okra, sweet potato greens, purple hull peas and purslane. The content ranged from 1.3 to 31.8 mg/100 g with the highest content in kale and lowest content in purslane.

In present study, the determination of quercetin in some fruit samples which include rose hip (*Rosa canina*), nettle (*Urtica dioica*), terebinth (*Terebinthina chica*) and purslane (*Portulace oleracea*) was proposed. For this purpose, 30 mL extraction solution (0.2 M HCl + 5/6 dilute methanol + 0.17 M ascorbic acid) was used for 5 g of each sample. Then, the quercetin concentrations in these extracts were analyzed by using HPLC-MS with positive electrospray. Before the determination of quercetin in these samples, the experimental conditions such as injection volume, flow rate and column temperature were optimized with standard quercetin solution.

EXPERIMENTAL

Quercetin hydrate was purchased from Fluka (Buchs, Switzerland). Acetonitrile, formic acid, HCl, methanol and ascorbic acid were purchased from Merck (Darmstadt, Germany). All used solvents were of HPLC grade and other used chemicals were of analytical grade. Ultrapure water was obtained from water purification system (Millipore Direct Q). A stock solution of QU (500 mg L^{-1}) were prepared in acetonitrile and its diluted solutions in the range of 0.8 to 100 mg L^{-1} were freshly prepared from this stock solution in every 2 weeks and stored in refrigerator.

An Agilent 1200 HPLC-MS system was used for the quantification of quercetin. The HPLC-MS system is consisting of an autosampler, a binary pump, a temperature controlled column oven, coupled to an Agilent 1200 MS detector operated in selected ion monitoring (SIM) and SCAN mode equipped with electrospray positive ionization. Zorbax Eclipse XDB-C 18 (4.6 mm × 150 mm, 5 µm) was used as a HPLC column. The analysis was performed using a solvent system including water:methanol:acetonitrile:formic acid (52:42:5:1). The flow rate, injection volume, fragmentor potential and column temperature were used as 0.6 mL min^{-1}, 5 µL, 140 V and 30 ºC, respectively, which were optimized for 25 mg L^{-1} quercetin (Table-1).

The HPLC effluent entered the mass spectrometer through an electrospray capillary set at 4000 V. Nitrogen was used both as drying gas and vaporizer gas at 300 and 500 ºC temperatures, respectively. Drying gas flow was adjusted to 6 L min^{-1} (Table-1).

TABLE-1
OPTIMUM CONDITIONS FOR HPLC-MS PARAMETERS

Mobile phase	Water: methanol: acetonitrile: formic acid (52:42:5:1).
Mobil phase flow rate	0.6 mL min^{-1}
Column	Zorbax Eclipse XDB-C_{18} (4.6 mm × 150 mm, 5 µm)
Column temperature	30 ºC
Fragmentor potential	140 V
Injection volume	5 µL
Nebulizer (N) pressure	60 psi
Drying gas flow	6.0 L min^{-1}
Drying gas temperature	300 ºC
Vaporizer temperature	500 ºC
Capillary voltage	4000 V

Optimization of HPLC parameters: In order to optimize conditions related with HPLC-MS system, the effect of flow rate of mobile phase, injection volume, column temperature and fragmentor potential on chromatogram of quercetin were examined. In the each studied parameter, other parameters were used as their optimum values for quercetin solution of 25 mg L^{-1} in acetonitrile. Flow rates, injection volumes and column

temperatures were changed in the range of 0.4-1.0 mL min^{-1}; 5-40 µL and 20-55 ºC, respectively, for their optimization. In the MS system, the quercetin solutions were studied by changing the fragmentor potential in the range of 10-200 V for the optimization of fragmentor potential. Other applied conditions were summarized with obtained optimum parameters in Table-1.

Plant and fruit materials and sample preparation: Four different samples of rose hip (*Rosa canina*) and nettle (*Urtica dioica*) and three different samples of purslane (*Portulace oleracea*) were collected from the region of Elazig (Turkey) during their growing season in 2008. Further, three different samples of terebinth (*Terebinthina chica*) were purchased from a seller of herbs market in Elazig. These samples were washed carefully with pure water and dried at atmospheric temperature. Then, 5 g of each sample was grinded at an agate mortar and the extraction steps were applied to the sample at the optimum conditions.

For the extraction of quercetin from samples, 30 mL extraction solution which was formed the mixture of 5 mL of 1.2 M HCl, 25 mL of methanol and 0.9828 g ascorbic acid was added to 5 g of each dried and powdered plant. Then, the mixture was stirred at 90 ºC under reflux by Soxhlet extraction apparatus for 2 h to obtain aglycons by hydrolysis of the flavonol glycosides[33]. The extracts were cooled to room temperature and filtered by filter paper. The each filtrate was diluted to 30 mL with extraction solution and then filtered through a 0.45 µm filter (for terebinth a 0.20 µm filter was used). The obtained final solutions were analyzed with HPLC-ESI-MS system using optimum conditions. The scheme of optimized extraction procedure was given in Fig. 1.

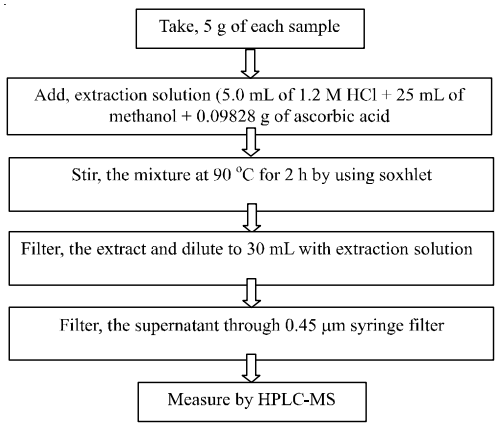


Fig. 1. Scheme of extraction procedure

In order to obtain linear calibration plot, various quercetin solutions in the concentration range of 0.8-100 mg L^{-1} were prepared and analyzed at optimum conditions by using HPLC-MS system. LOD and LOQ were determined by injection of a solution of quercetin (0.4 mg L^{-1}) with smallest detectable peak to column for five times.

In order to obtain the repeatability of the method, a standard solution in concentrations of 25 mg L^{-1} quercetin was injected to column for 5 times. Then, the relative standard deviation (RSD) values were calculated and evaluated for retention time and integration area.

The recovery of method was investigated by standard addition method for purslane samples. The concentrations of quercetin in four extraction solution of purslane with 30 mL were arranged as 0.0, 1.0, 3.0 and 6.0 mg L^{-1} respectively. For this aim, a proper amount of stock quercetin solution was added to each extract prior to HPLC analysis. Then, their chromatograms were obtained at the optimum conditions.

RESULTS AND DISCUSSION

Optimization of method: The parameters that are thought to affect the measurement steps in the analytical scheme were examined by using the quercetin solutions of 25 mg L^{-1}. In the evaluation of optimum conditions, the peak area, peak symmetry and abundance were taking into consideration. Furthermore, each parameter was optimized by using the other optimum conditions. In order to obtain an effective separation in chromatographic methods, firstly the best suitable column type and mobile phase should be selected. Our preliminary studies showed that the best results were obtained when we used C_{18} columns. Thus, Zorbax Eclipse XDB-C_{18} (4.6 mm × 150 mm, 5 µm) was used a HPLC column in this study. Moreover, different mobile phases were tried and best results were obtained with water:methanol: acetonitrile:formic acid in ratio 52:42:5:1, respectively.

In order to see the effect of flow rate on peak shape and peak area of quercetin, the chromatograms for 25 mg L^{-1} quercetin were recorded by using different flow rates of mobile phase. The best results were obtained when flow rate was used as 0.6 mL min^{-1}. At the low flow rates, the peak shape was broadening and retention time was found to be very high. Thus, when the peak shape, retention time and also peak area were taking into consideration, the flow rate of 0.6 mL min^{-1} was found as an optimum value.

To obtain optimum fragmentor potential, the chromatograms of 25 mg L^{-1} quercetin were recorded by using fragmentor potantial in the range of 10-200 V. From the results obtained, peak areas were found as maximum values between 120 and 180 V. Due to obtaining maximum value in abundance at 140 V, this fragmentor potential was preffered as optimum value.

In order to obtain the optimum injection volume, the chromatograms of quercetin with various injection volumes were recorded. These chromatograms indicated that the best result was obtained for 5 µL of injection volume. Furthermore, the peak symmetry was changed when the injection volume was higher than 5 µL. Thus, 5 µL was chosen as the optimum injection volume.

Finally, the column temperature was optimized. For the determination of optimum column temperature, the separately quercetin solutions at the same concentration were examined by using different column temperatures between 20 and 55 ºC. From the obtained peak areas, the temperature of 30 ºC was chosen as optimum and this temperature was used for subsequent studies. The obtained optimum conditions and HPLC-MS parameters were summarized in Table-1.

Analytical performance: The validation of this method for quercetin was performed by investigation of different parameters like linearity, limit of detection (LOD) and limit

of quantification (LOQ), precision and accuracy. The chromatographic responses *vs*. various concentration of quercetin were recorded using optimum conditions (flow rate: 0.6 mL min^{-1}; fragmentation potential: 140 V; injection volume: 5 mL and column temperature: 30 ºC). The obtained chromatograms for some standard quercetin solutions were given in Fig. 2. The inset of Fig. 2 shows a plot of peak area *vs*. quercetin concentration. From this figure, a linear relationship between quercetin concentration and peak area was obtained over the concentration range 0.8-100 mg L^{-1}. The linearity of this method was described by below equation.

$Y = 10665X - 3958.5$ $R^2 = 0.9996$ for 0.8-100 mg L^{-1} quercetin

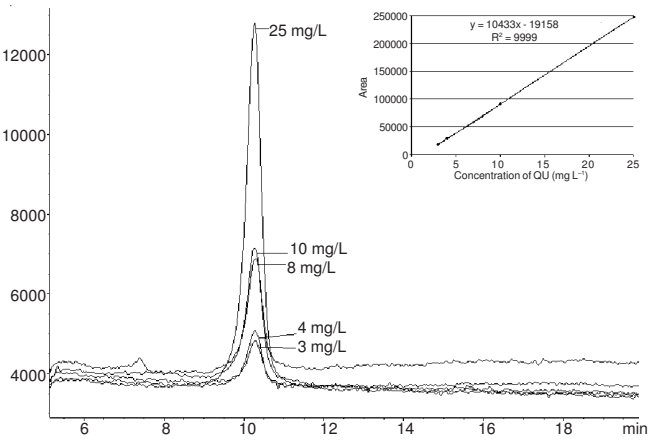


Fig. 2. Chromatograms of quercetin with different concentrations (10, 25, 50 and 100 mg L^{-1}). Inset shows the plot of peak area *vs*. the concentration of quercetin

The sensitivity of the method was determined with respect to LOD and LOQ. The standard stock solutions were diluted with acetonitrile to provide serial solutions with their concentrations decreasing to the smallest detectable peaks. LOD was found to be 0.1 mg L^{-1}, while LOQ was found to be 0.4 mg L^{-1}. These results indicate that the sensitivity of proposed method is very good for determination of quercetin in natural products.

The repeatability of the chromatographic method was tested using a standard solution in concentrations of 25 mg L^{-1} quercetin. The solutions were injected five times and RSD values were calculated for both retention time and integration area and considered as a measure of precision. RSD was calculated as 3.5 % and 2.4 % for peak area and retention time, respectively, which demonstrated the good precision of this method.

Validation of the method was achieved by determination of the recovery during standard addition. The SIM chromatograms related with the standard additions method for purslane were given in Fig. 3. The observed peaks between 2.0-9.0 min in this chromatogram are attributed to other polyphenols including quercetin. Furthermore, the slope of calibration curves was compared with the slopes obtained by the standard addition method (Inset of Fig. 3). Because the slopes of calibration curve and standard addition method are identical, it was concluded that the calibration curve could be used for the quantitative analysis. The slopes of both calibration curves were found very similar to each other. Thus, we can say that, any important interference effect on the determination of quercetin in purslane extract was not observed. Furthermore, the recoveries at different quercetin levels were calculated from the results of standard addition method. The recoveries were found at least 90 %. The results showed that this method was adequate and appropriate for the quercetin analysis.

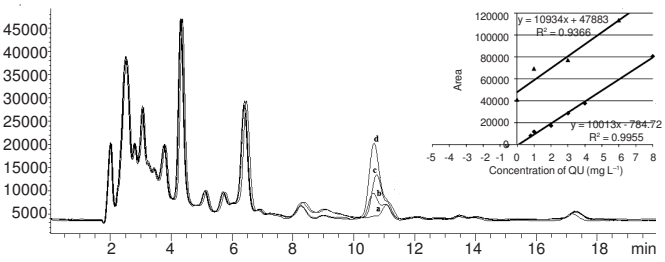


Fig. 3. HPLC-MS chromatograms obtained from standard addition method for purslane extraction: a) purslane extract b) a + 1 mg L^{-1} quercetin c) a+3 mg L^{-1} quercetin d) a+6 mg L^{-1} quercetin. Inset shows calibration curve for standard solution of quercetin and standard addition method

Applications: In order to indentification of the quercetin in aglycone form, HPLC-MS analysis were performed in positive mode. In the selected ion monitoring (SIM) chromotograms, a main peak was observed at precursor ion m/z 303. This peak was attributed to the characteristic peak of quercetin in aglycone form. Yang and coworkers also reported that a peak at m/z 303 obtained from MS spectrum of lotus petals in positive ion mode demonstrated the identification of quercetin[20].

It is well known that, quercetin is generally found as its glycosidic form in plants. However, free flavonol aglycones can be produced from their glycosides via hydrolysis of the glycosidic bond by enzymes or acidic conditions. In order to obtain an aglycone form of quercetin, a mixture of 5 mL of 1.2 M HCl, 25 mL of methanol and 0.9828 g ascorbic acid was used as an extraction solution for 5 g sample. The extracts obtained from Rose hip, nettle, purslane and terebinth samples were analyzed for the determination of quercetin using HPLC-MS method. The sample solutions were injected directly and separated under the optimized conditions. On the other hand, standard addition methods were applied for all samples. For example, the chromatograms obtained from standard addition method and a related calibration curve for extract of purslane was shown in Fig. 3. The content of quercetin in these samples was determined by the corresponding regression equation and obtained results were summarized in the Table-2. It shows that the content of quercetin varied from 27 to 35 (average 31) mg kg^{-1} for rose hip, 16 to 23 (average 19) mg kg^{-1} for nettle, 36 to 42 (average 39) mg kg^{-1} for purslane and 8-13 (average 10) mg kg^{-1} for terebinth on the basis of dry weight.

When quercetin results obtained from this method were compared with that obtained from literature, the quercetin levels were found in the range of reported values. For example, Huang and coworkers analyzed the phenolic composition of vegetables commonly consumed by African Americans in the southeast United States with HPLC-MS[21]. The quercetin was found in collard greens, mustard greens, kale, okra, sweet potato greens, purple hull peas and purslane in the range from 13 to 318 mg kg^{-1}. The highest and lowest quercetin content was found in kale and purslane, respectively. However, in our

study the highest quercetin level was found in purslane (42 mg kg^{-1}) while the lowest level was found in terebinth (8 mg kg^{-1}). In another study, quercetin content of *Urtica sp.*, *Rosa canina* (rosehip), *Salvia officinalis* (sage), *Tilia platyphyllos* (linden flower), black tea, *Daucus carota L. spp sativus* (violet carrot juice), grape molasses, honey and tarhana were determined by HPLC with UV detection[34]. The quercetin content of liquid samples were found to be 34.8, 21.7, 27.2, 16.7, 83.7 and 1692 mg L^{-1} for black tea, linden flower, sage, rosehip, violet carrot juice and grape molasses, respectively. The quercetin contents of solid samples were found to be 59.2 for tarhana and 8.7 for *Urtica sp.*. However, it was not found in honey samples.

TABLE-2
RESULTS FOR DETERMINATION OF QUERCETIN IN ROSE HIP, NETTLE, PURSLANE AND TEREBINTH SAMPLES (n=3)

Sample	Amount of quercetin found (mg kg^{-1})
Rose hip 1	29 ± 2
Rose hip 2	33± 3
Rose hip 3	27± 3
Rose hip 4	35± 4
Rose hip average	31 ± 3
Nettle 1	18 ± 1
Nettle 2	20 ± 2
Nettle 3	16 ± 2
Nettle 4	23 ± 2
Nettle average	19 ± 2
Purslane 1	39 ± 3
Purslane 2	42 ± 4
Purslane 3	36 ± 4
Purslane average	39 ± 4
Terebinth 1	9 ± 0.5
Terebinth 2	13 ± 1
Terebinth 3	8 ± 1
Terebinth average	10 ± 1

This method was successfully applied to determination of quercetin in the plant and fruits samples after their extraction with a mixture of 5 mL of 1.2 M HCl, 25 mL of methanol and 0.9828 g ascorbic acid for 5 g sample. As a result, the optimized HPLC-MS method can be successively used for the determination of quercetin in rose hip, purslane, nettle and terebinth.

Conclusion

The presented study describes a simple and optimized HPLC-MS analytical method for the determination of quercetin in four different plant samples, which are rose hip, nettle, purslane and terebinth. For achieving a good separation, column and mobile phase was chosen as Zorbax Eclipse XDB-C 18 (4.6 mm × 150 mm, 5 µm) column and water: methanol:acetonitrile:formic acid in ratio 52:42:5:1, respectively. The optimized conditions were found to be 0.6 mL min^{-1}, 5 µL, 30 ºC and 140 V for flow rate of mobile phase, injection volume, column temperature and fragmentor potential, respectively. There are a lot of papers on the determination of this important flovonoid using chromatographic methods. However, it was changed one of some parameters such as extraction methods, detection modes, plant material types or their growing place and optimization parameters like our study in these studies. In our study, four different plants and fruits which were grown in Elazig (Turkey) were selected as samples and a mixture of 5 mL of 1.2 M HCl, 25 mL of methanol and 0.9828 g ascorbic acid was used as an extraction solution. Thus, the determination of quercetin in four different plants was easily achieved by using HPLC-MS method. This method was validated in terms of linearity, precision, accuracy and sensitivity using these optimum parameters. The calibration curves were found to be linear in the range of 0.8-100 mg L^{-1} quercetin. The relative standard deviation for retention time and peak area of 25 mg L^{-1} quercetin was found to be 2.4 % and 3.5 % respectively, indicated that this method has a good repeatability.

REFERENCES

1. B. Portet, N. Fabre, R. Rozenberg, J.-L. Habib-Jiwan, C. Moulis and J. Quetin-Leclercq, *J. Chromatogr. A*, **1210**, 45 (2008).
2. G. Dinelli, A. Bonetti, M. Minelli, I. Marotti, P. Catizone and A. Mazzanti, *Food Chem.* **99**, 105 (2006).
3. U. Justesen and P. Knuthsen, *Food Chem.*, **73**, 245 (2001).
4. G. Seelinger, I. Merfort, U. Wolfle and C.M. Schempp, *Molecules*, **13**, 2628 (2008).
5. J. Lameira, C.N. Alves, V. Moliner and E. Silla, *Eur. J. Med. Chem.*, **41**, 616 (2006).
6. B.Q. Li, T. Fu, W.H. Gong, N. Dunlop, H.F. Kung, Y.D. Yan, J. Kang and J.M. Wang, *Immunopharmacology*, **49**, 295 (2000).
7. S.J. Semple, S.F. Nobbs, S.M. Pyke, G.D. Reynolds and R.L.O. Flower, *J. Ethnopharmacology*, **68**, 283 (1999).
8. A. Bravo and J.R. Anacona, *Transition Met. Chem*., **26**, 20 (2001).
9. H.C. Hung, K.J. Joshipura, R. Jiang, F.B. Hu, D. Hunter, S.A. Smith-Warner, G.A. Colditz, B. Rosner, D. Spiegelman and W.C. Willett, *J. Nat. Cancer Inst.*, **96**, 1577 (2004).
10. B. Halliwell, *Lancet*, **344**, 721 (1994).
11. M.G.L. Hertog, E.J.M. Feskens, P.C.H. Hollmann, M.B. Katan and D. Kronthout, *Lancet*, **342**, 1007 (1993).
12. N. Kumar, P. Bhandari, B. Singh and S.S. Bari, *Food Chem. Toxicol.*, **47**, 361 (2009).
13. R. Ferraresi, L. Troiano, E. Roat, E. Lugli, E. Nemes, M. Nasi, M. Pinti, M.I.G. Fernandez, E.L. Cooper and A. Cossarizza, *Free Radic. Res.*, **39**, 1249 (2005).
14. J.M. Pauff and R. Hille, *J. Nat. Prod.*, **72**, 725 (2009).
15. M. Biesaga and K. Pyrzynska, *Crit. Rev. Anal. Chem.*, **39**, 95 (2009).
16. A.T. Jan, M.R. Kamli, I. Murtaza, J.B. Singh, A. Ali and Q.M.R. Haq, *Food Rev. Int.*, **26**, 302 (2010).
17. L. Reddy, B. Odhav and K.D. Bhoola, *Pharmacol. Therap.*, **99**, 1 (2003).
18. D.G. Watson and E.J. Oliveira, *J. Chromatogr. B*, **723**, 203 (1999).
19. E. Aguirre-Hernández, M.E. González-Trujano, A.L. Martínez, J. Moreno, G. Kite, T. Terrazas and M. Soto-Hernández, *J. Ethnopharmacol.*, **127**, 91 (2010).
20. R.-Z. Yang, X.-L. Wei, F.-F. Gao, L.-S. Wang, H.-J. Zhang, Y.-J. Xu, C.-H. Li, Y.-X. Ge, J.-J. Zhang and J. Zhang, *J. Chromatogr. A*, **1216**, 106 (2009).
21. Z. Huang, B. Wang, D.H. Eaves, J.M. Shikany and R.D. Pace, *Food Chem.*, **103**, 1395 (2007).
22. D.W. Jeffery, M. Parker and P.Q. Smith, *Aust. J. Grape Wine Res.*, **14**, 153 (2008).
23. H.R. Goo, J.S. Choi and D.H. Na, *Arch. Pharmacal. Res.*, **32**, 201 (2009).
24. B. Sultana and F. Anwar, *Food Chem.*, **108**, 879 (2008).
25. G. Elgin, S. Konyalioglu and E. Kilinc, *J. Liq. Chromatogr. Rel. Technol.*, **32**, 432 (2009).
26. K.H. Park, P. Garm de Oca, G.A. Bonello, K.-J. Lee and K. Dabrowski, *Aquacult. Int.*, **17**, 537 (2009).
27. Q. Zhang and H. Cui, *J. Sep. Sci.*, **28**, 1171 (2005).
28. Y. Sun, T. Guo, Y. Sui and F. Li, *J. Sep. Sci.*, **26**, 1203 (2003).
29. D. Zielinska, L. Nagels and M.K. Piskula, *Anal. Chim. Acta*, **617**, 22 (2008).
30. P. Xiao, F. Zhao and B. Zeng, *Microchem. J.*, **85**, 244 (2007).
31. H.F. Askal, G.A. Saleh and E.Y. Backheet, *Talanta*, **39**, 259 (1992).
32. Z.N. Coleska, L.J. Klisarova, L.J. Suturkova and K. Dorevski, *Anal. Lett.*, **29**, 97 (1996).
33. Q. Tokusoglu, M.K. Unal and Z. Yildirim, *Acta Chromatogr.*, **13**, 196 (2003).
34. S. Karakaya and S.N. El, *Food Chem.*, **66**, 289 (1999).